

HEMAGGLUTINATING ACTIVITY IN EXTRACTS OF SOME MARINE MEXICAN ALGAE

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ABSTRACT — Eleven algal species collected along the coast of the states of Guerrero and Oaxaca, Mexico, were analyzed to detect agglutinating activity in human blood erythrocytes in A⁺, B⁻ and O⁺ groups. Aqueous extracts were prepared and frozen until analysis. Microtitration tests were performed with serial dilutions of algal extract and with a constant volume of human erythrocytes at 2%. They were left to settle, and after two hours, observed under a microscope. Out of 15 samples analyzed, 20% showed positive agglutination. The active species were *Codium giraffa*, *Hypnea spinella* and *Ulva lactuca*. This is the first report of agglutinating activity from Mexican algae, and for *Codium giraffa* and *Hypnea spinella*.

RÉSUMÉ — Onze espèces d'algues récoltées sur la côte des états de Guerrero et d'Oaxaca, au Mexique, ont été étudiées dans le but de tester leur activité agglutinante vis à vis des érythrocytes du sang humain des groupes A⁺, B⁻ et O⁺. Des extraits aqueux ont été préparés et congelés jusqu'à leur étude. Des tests de microtitration ont été conduits en utilisant des dilutions en série des extraits d'algues et un volume constant d'une suspension d'érythrocytes humains à 2%. Après deux heures, l'agglutination a été observée au microscope. Sur les 15 échantillons étudiés, 20% ont montré une activité agglutinante. Les espèces dont les extraits ont présenté une activité provenaient de *Codium giraffa*, *Hypnea spinella* et *Ulva lactuca*. L'existence d'une possible activité agglutinante est signalée pour la première fois chez des algues mexicaines d'une part et chez *Codium giraffa* and *Hypnea spinella* d'autre part. (Traduit par la Rédaction)

KEY WORDS --- Hemagglutination, agglutinins, agglutinating activity, seaweeds, Mexico.

INTRODUCTION

Lectins are proteins or glycoproteins capable of binding to simple and complex sugars. They have been isolated mainly from species of the Leguminosae (Sharon & Lis, 1972). Boyd *et al.* (1966) were the first to test alga extracts against human erythrocytes; they found positive responses for nine species. Blunden *et al.* (1975) found that extracts from the red alga *Ptilota plumosa* (Huds.) C. Agardh showed specificity against B type human blood erythrocytes. Rogers & Blunden (1980) isolated

and partially characterized the active principle which was a protein with two subunits, one with a molecular weight of 65 kD and the other 170 kD. Subsequent studies of algal agglutinant agents include Rogers & Hori (1993), Ainouz *et al.* (1992) and Bird *et al.* (1993).

MATERIALS AND METHODS

Algal samples were collected at Cacaiotepec, Puerto Escondido, Carrisalillo, Punta de Zicatela, Playa Zipolite and Punta Arena in the State of Oaxaca and Acapulco in the State of Guerrero, Mexico (Fig. 1), and were kept frozen until their use. Samples were thawed at room temperature, washed with distilled water and manually cleaned of epiphytes. Ten grams of algal material was ground in a mortar with 10 ml of isotonic saline solution (pH 6.8), to make a uniform homogenate. This was strained through muslin to remove larger particles. The filtrate was placed in 15 ml polypropylene tubes and was centrifuged at 1 000 g for 10 minutes. The supernatant was then collected using a disposable syringe and filtered through Millipore[®] equipment using 22 µm nitrocellulose filters. The extract obtained was stored in 60 ml inert plastic flasks and frozen at -6° C until used (Muñoz *et al.*, 1985). The blood cell solution was prepared following the technique used by Bennet (1976). Agglutination tests were done in microtiter-plates using serial double dilution of 100 µl of saline serum and 100 µl of algal extracts, adding 100 µl volume of 2 % erythrocytes. The plates were gently shaken for 15 seconds and left at room temperature for 2 hours, and agglutination of erythrocytes was observed under a microscope (Fabregas *et al.*, 1984). The titre was expressed as the reciprocal of the highest dilution showing positive results (Ainouz & Sampaio, 1991).

RESULTS AND DISCUSSION

Eleven algal species were tested: 5 Chlorophyta: *Chaetomorpha antennina* (Bory) Kützing, *Codium giraffa* Silva, *Enteromorpha intestinalis* (Linnaeus) Link, *Halimeda discoidea* Decaisne and *Ulva lactuca* Linnaeus; 4 Phaeophyta: *Chnoospora minima* (Hering) Papenfuss, *Padina durvillaei* Bory, *Padina gymnospora* (Kützing) Sonder and *Sargassum liebmanii* J. Agardh; and 2 Rhodophyta: *Amphiroa beauvoisii* Lamouroux and *Hypnea spinella* (C. Ag.) Kützing.

Of the 15 algal samples tested (Table 1), agglutinating activity was observed in *Ulva lactuca*, *Codium giraffa*, and *Hypnea spinella*. *Codium giraffa* showed activity against all the erythrocytes bioassayed with a titre value of 2⁷. Previously, Blunden *et al.* (1975) and Gilboa-Garber *et al.* (1988), reported that *Ulva lactuca* showed agglutinating activity to human erythrocytes as we found in the present study. Activity in the extracts of *Ulva lactuca* was only found in material collected at Punta Arena, but not in the material collected at Carrisalillo. Both places are in the coast of the state of Oaxaca. This difference may be due to local variation of activity related to ecological conditions, as suggested by Fabregas *et al.* (1985) and by Ingram (1985). The highest

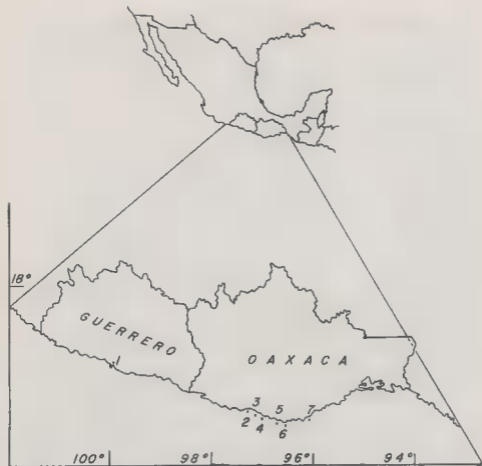


Fig. 1. Location of collection sites. 1 Acapulco, 2 Cacalotepec, 3 Puerto Escondido, 4 Carrisalillo, 5 Punta de Zicatela, 6 Playa Zipolite, 7 Punta Arena.

titres were found in extracts of *Ulva lactuca* and *Hypnea spinella*, both against type O positive blood. For extracts of *Ulva lactuca* the titres that agglutinated groups A and B were of 2^3 and 2^1 respectively, while *Hypnea spinella* had a 2^7 and 2^6 range for the same types of blood.

Species of *Codium* previously have shown positive hemagglutination (Boyd *et al.*, 1966; Blunden *et al.*, 1975; Fabregas *et al.*, 1988; Chiles and Bird, 1989). We report, for the first time, the agglutinating activity from *Codium giraffa* from Mexican Tropical Pacific. Considering that proteolytic enzymes were not used to help agglutination (Rogers *et al.*, 1982; Kamiya *et al.*, 1982; Hori *et al.*, 1988), the titres obtained were high compared with those reported by Boyd *et al.* (1966), Fabregas *et al.* (1985) and Ainouz & Sampaio (1991) where untreated erythrocytes were used.

Division and species	Locality	Blood group		
		O ⁺	A ⁺	B ⁺
Chlorophyta				
<i>Chaetomorpha antennina</i>	Punta de Zicatela	-	-	-
<i>Codium giraffa</i>	Punta de Zicatela	2 ⁷	2 ⁷	2 ⁷
<i>Enteromorpha intestinalis</i>	Acapulco	-	-	-
<i>Halimeda discoidea</i>	Playa Zipolite	-	-	-
<i>Ulva lactuca</i>	Punta Arena	2 ⁸	2 ³	2 ¹
<i>Ulva lactuca</i>	Carrisalillo	-	-	-
Phaeophyta				
<i>Chnoospora minima</i>	Cacalotepec	-	-	-
<i>Padina durvillaei</i>	Acapulco	-	-	-
<i>Padina durvillaei</i>	Punta de Zicatela	-	-	-
<i>Padina gymnospora</i>	Acapulco	-	-	-
<i>Padina gymnospora</i>	Punta de Zicatela	-	-	-
<i>Sargassum liebmanii</i>	Cacalotepec	-	-	-
Rhodophyta				
<i>Amphiroa beauvoisii</i>	Punta de Zicatela	-	-	-
<i>Amphiroa beauvoisii</i>	Cacalotepec	-	-	-
<i>Hypnea spinella</i>	Puerto Escondido	2 ⁸	2 ⁷	2 ⁶

Tableau 1. Agglutinant activity of algal extracts against blood groups O, A and B erythrocytes. "-" means a non positive test. 2ⁿ is the titre measured.

Given the spatial and seasonal abundance of *Ulva lactuca*, *Codium giraffa* and *Hypnea spinella* along the Mexican tropical Pacific coast, further research is needed on the effects of the seasonal and reproductive states to possibly explain why a species from one place shows activity, but not if collected from a nearby site.

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